Supplemental Information

Scalable inhibitors of the Nsp3-Nsp4 coupling in SARS-CoV-2

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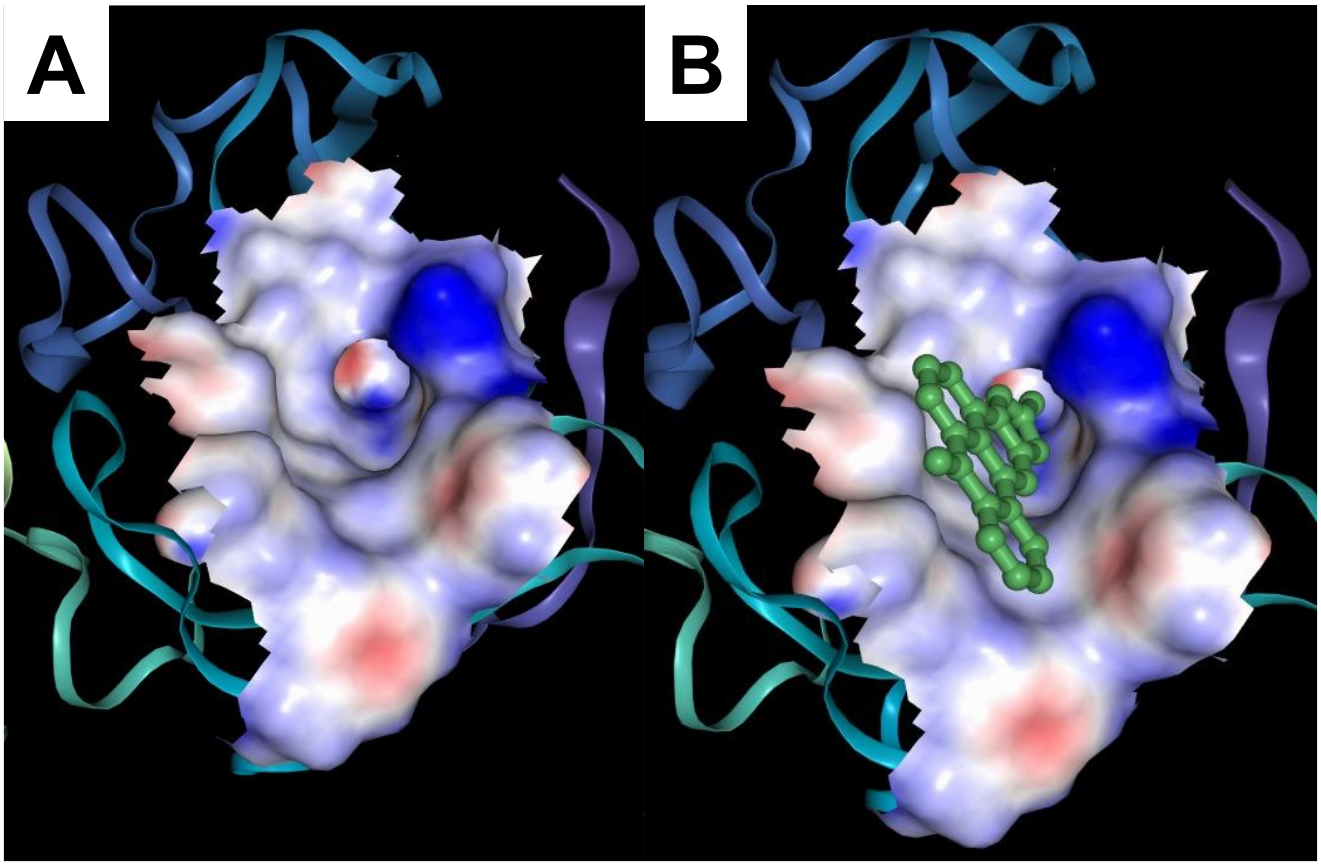
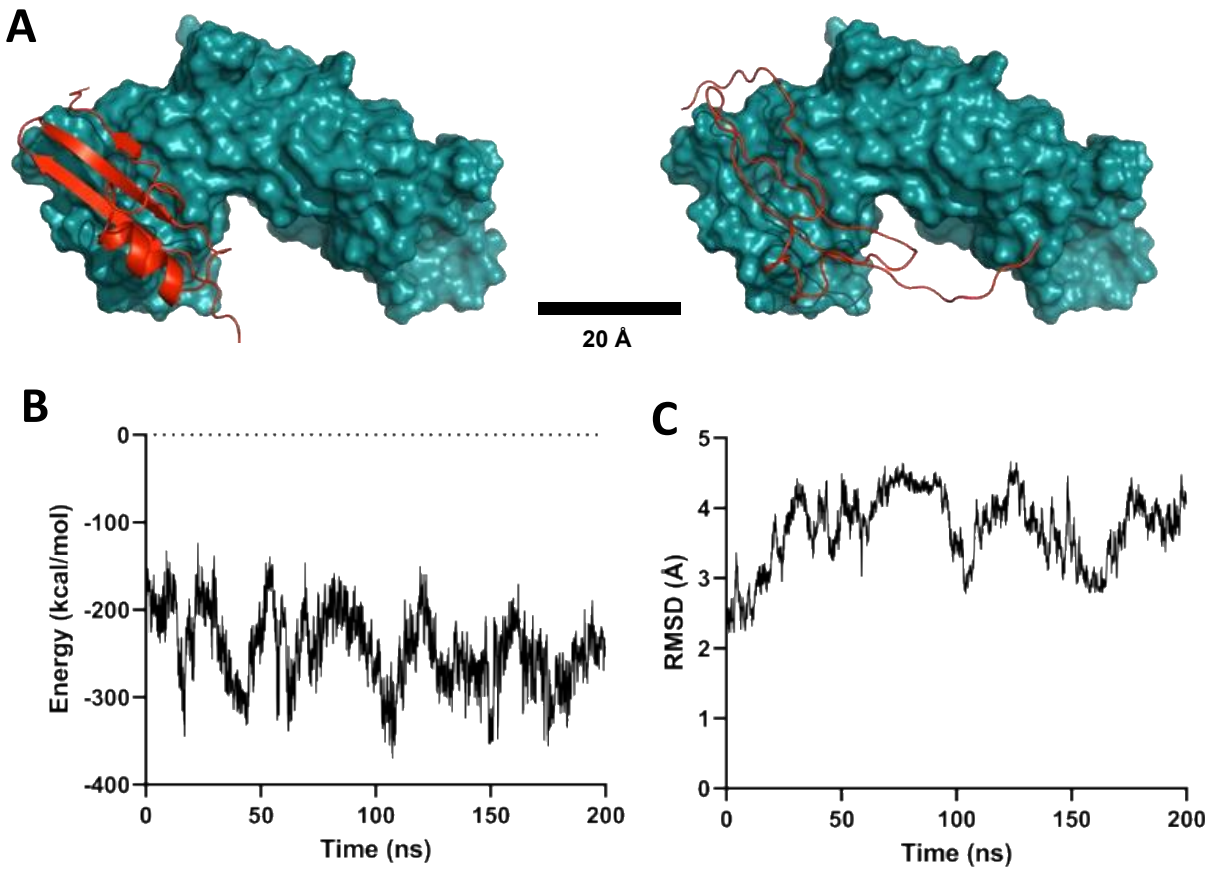
KEYWORDS: SARS-CoV-2, molecular docking, drug discovery, nonstructural proteins

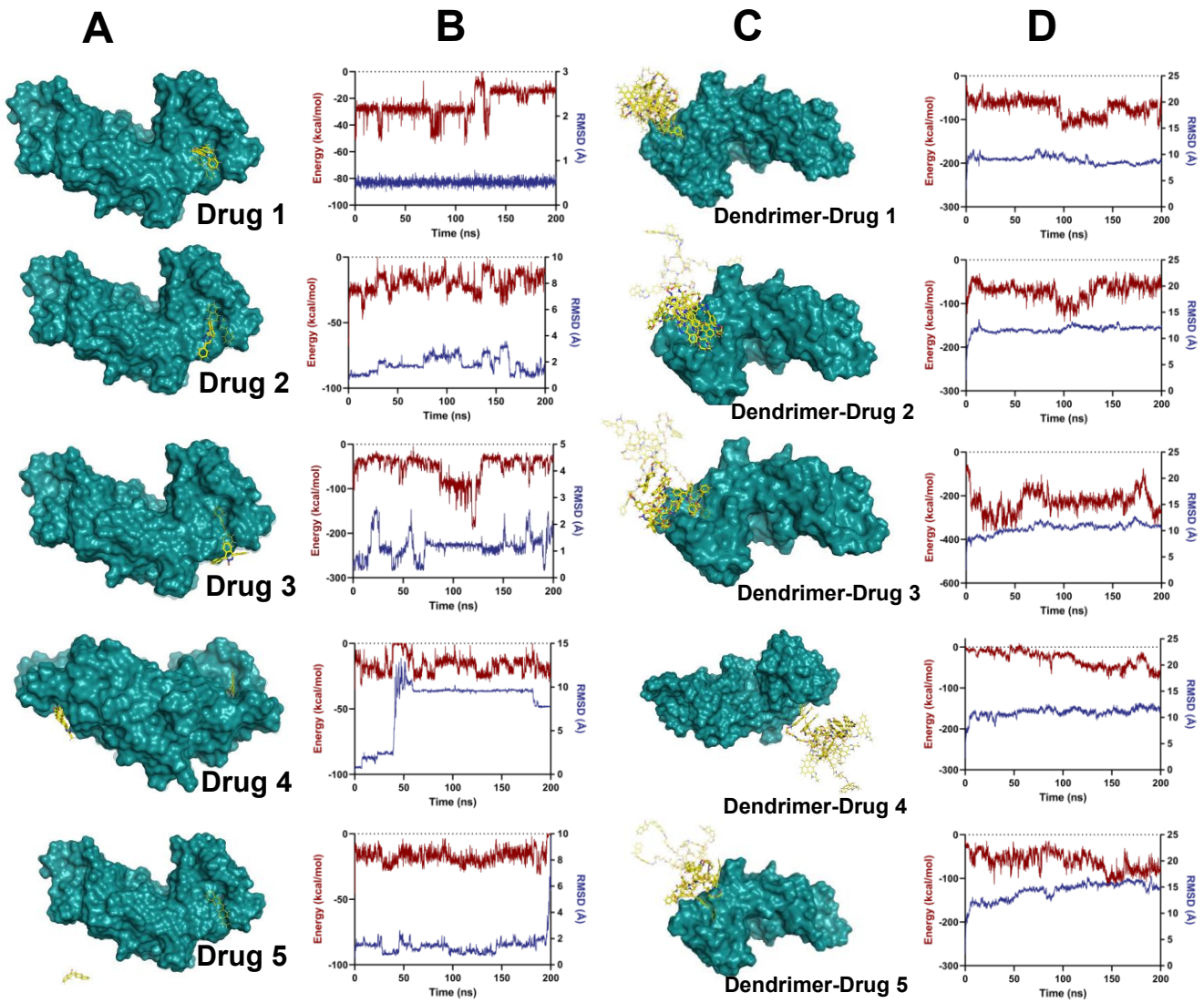
Supplemental Information contains:



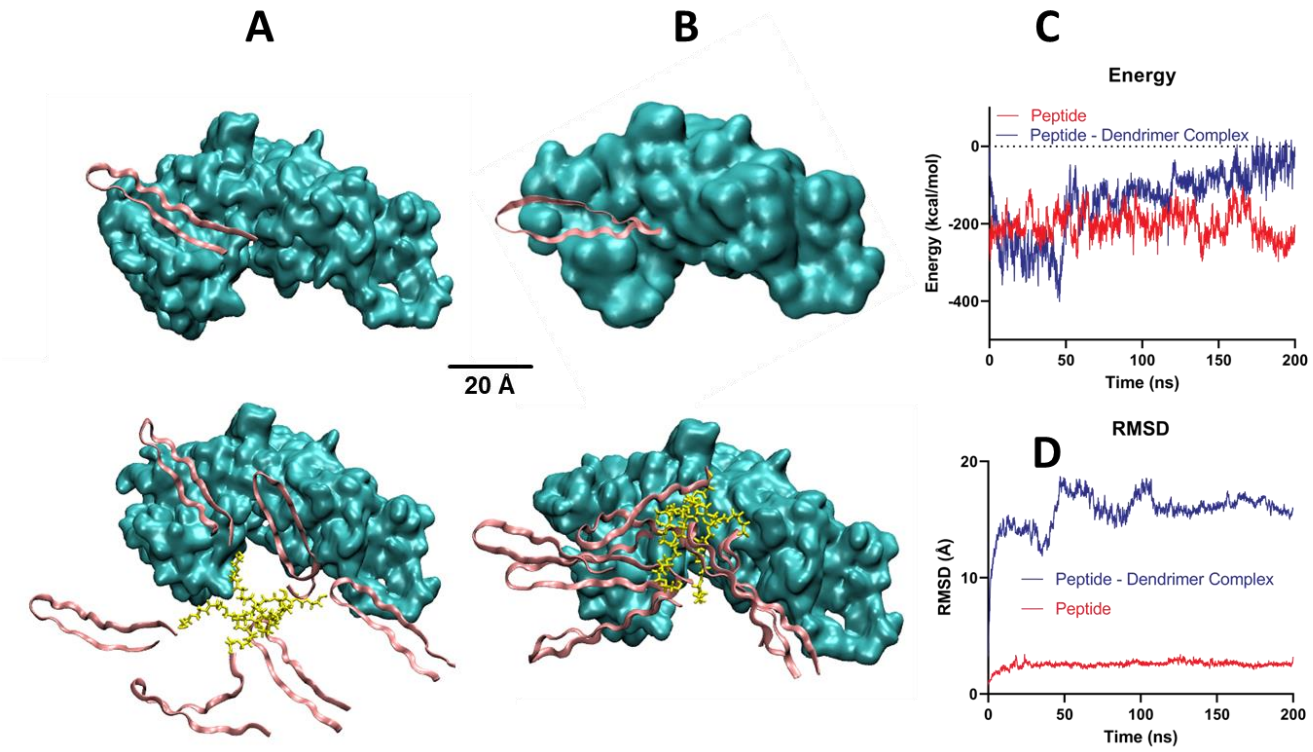
SI Figure 1. Electrostatic Charge Maps of the Protein-Ligand Complex Post AutoDock Vina® Screening. A) Electrostatic charge map of the binding pocket on SARS-CoV-2 Nsp4LLL and B) with a ligand. The residues which line the cavity are 65, 67-68, 90-92, 101, 103, 106, 110, 118-120.



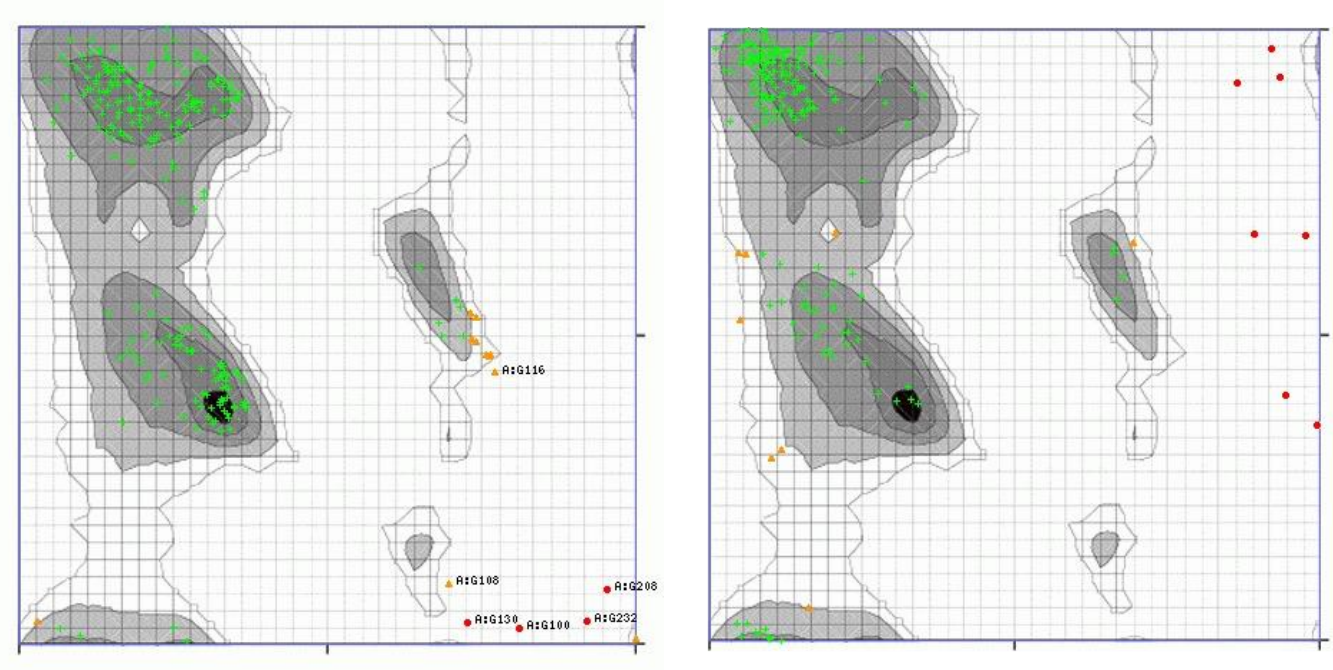
SI Figure 2. Control Simulation Data. (A) Nsp3 (segment)-Nsp4 complex simulations of SARS-CoV-2 proteins were executed. Nsp3 segment is shown in red cartoon and Nsp4 is shown in blue surface. Initial conformation shown to the left with the final complex after 200ns of production MD simulation shown on the right. (B) Binding energy between Nsp3 and Nsp4 vs. time. (C) RMSD of the peptide over time.



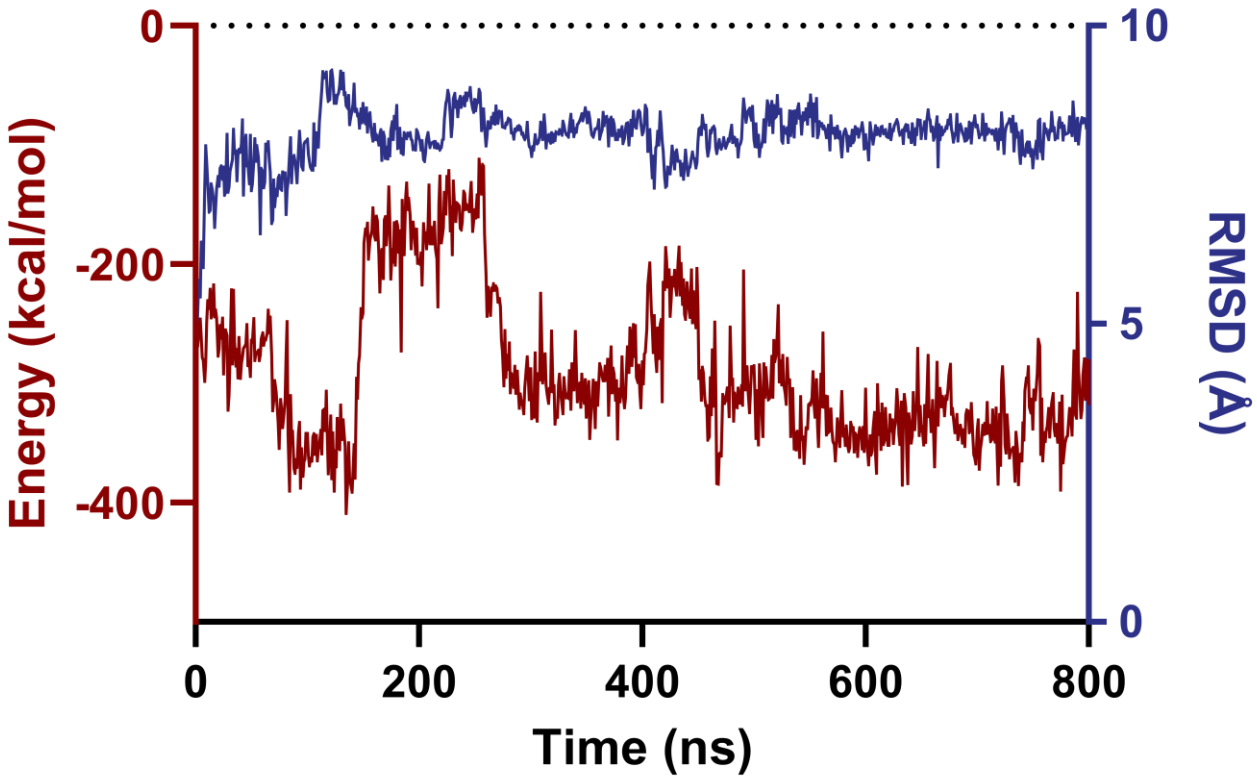
SI Figure 3. Drug and Dendrimer on Fixed Nsp4 Simulation Data. Column (A) Nsp4-Drug 1-5 interactions and (C) Nsp4-Dendrimer conjugated to Drug 1-5 simulations of SARS-CoV-2 were executed for 200ns, Energy and RMSD shown in column (B) and (D), respectively, with the initial state at 70% transparency and the final state shown as opaque. Binding energy and RMSD graphs are shown. The ligand is shown in yellow and Nsp4 is shown in blue.



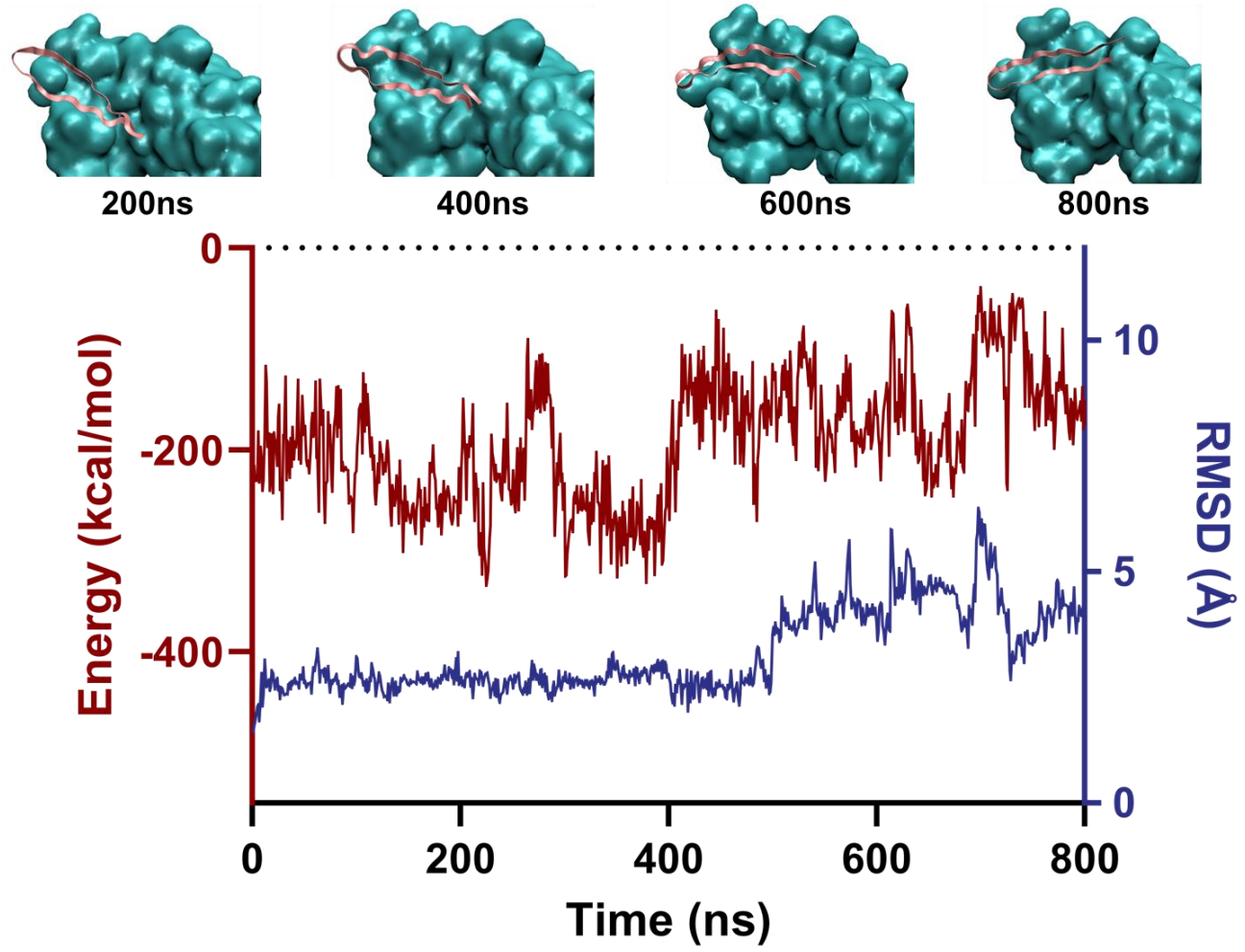
SI Figure 4. Peptide on Nsp4 Simulation Data. Peptide-Nsp4 complex and a Dendrimer Peptide-Nsp4 complex of SARS-CoV-2 were executed. Six of the same peptide was covalently attached in the dendrimer simulation with (A) showing the complex at 0ns, (B) showing the complex after 200ns, (C) showing the binding energy of the drug on Nsp4, and (D) showing the RMSD of the drug on Nsp4. The dendrimer base is shown in yellow, covalently attached peptides are shown in pink, and Nsp4 is shown in blue surface.



SI Figure 5. Ramachandran Plots of Fixed Nsp4LLL Simulation. Initial SARS-CoV-2 Nsp4LLL and the N and C terminal transmembrane domains as shown in Figure 1B. On the left analysis with the structure generated by Alphafold where 4 questionable observations shown as RED Circles (1.633%) and 11 preferred observations shown as BROWN Triangles 11 (4.490%). On the right, on the other hand, we have the structure when one end is rotated so that it can be inserted into a membrane with 5 questionable observations shown as RED Circles (1.736%) and 6 preferred observations shown as BROWN Triangles (2.083%). Once the simulation has started with this new membrane conformation, however, Nsp4 reaches 14 questionable observations (4.861%) and 11 preferred observations (3.819%).



SI Figure 6. Drug 3 Dendrimer Extended Simulation. Longer simulations of drug 3 dendrimer on Nsp4 were conducted for 800ns in order to observe stability (Energy (red), RMSD (blue)). Simulation showed that after 300ns, the dendrimer starts to stabilize and reach equilibrium with minor fluctuations in RMSD.



SI Figure 7. Peptide Extended Simulation. Longer simulations of the peptide on Nsp4 were conducted for 800ns in order to observe stability (Energy (red), RMSD (blue)). Snapshots at 200, 400, 600 and 800ns show the peptide roams around the binding pocket but remains stably bound over time.

SI Table 1. BLASTp Results from Comparison of Nsp3 and Nsp4 in All Coronaviruses to SARS-CoV-2. Breakdown of sequence identities in Nsp3, Nsp3Ecto, Nsp4, and Nsp4LLL across a variety of coronaviruses in comparison to the corresponding sequences in SARS-CoV-2 original strain. Also indicated are the annotated protein identifiers in NCBI corresponding to the conserved sequences.

Protein	SARS-CoV	MERS-CoV	NL63	OC43	HKU1	229E
NSP3	NSP3/ 75.82%	NSP3/ 30.54%	NSP3/ 28.16%	VP & NAR/ 29.52%	VP,NAR, TMDs/ 29.28%/	NSP3 28.69%
NSP3Ecto	70.73%	31.25%	36.67%	35.48%	31.67%	31.37%
NSP4	NSP4/80.00%	NSP4/39.96%	NSP4/29.20%	TMD2/42.53%	NSP4/43.57%	NSP4/27.27%
NSP4LLL	82.79%	40.32%	29.39%	42.35%	43.95%	28.51%

SI Table 2. Comparison of SARS-CoV-2 Variants in Spike Protein and Nsp4 Mutations. Unique changes (different amino acid or deletion) at each position were included for each variant in comparison to the original strain.

SARS-CoV-2 Variant	Unique Mutations on Nsp4	Unique Mutations on Spike Protein
Alpha	**0**	**10**
Beta	**0**	**11**
Gamma	**0**	**12**
Delta	**3 (V167L, A446V, T492I)**	**9**
Omicron	**4 (L264F, T327I, L438F, T492I)**	**45**

SI Table 3. Average Binding Energy & RMSD for All Simulations

	Drugs on Nsp4		Drugs on Nsp4 with Nsp3			Dendrons on Nsp4	
	E (kcal/mol)	RMSD (Å)	E drugs on nsp4 (kcal/mol)	E Nsp3 on Nsp4 (kcal/mol)	RMSD drugs (Å)	E (kcal/mol)	RMSD (Å)
d1	-24.42	0.52	-20.53	-191.13	6.09	-72.9	8.94
d2	-19.71	1.81	-48.22	-211.64	5.53	-70.18	11.74
d3	-52.62	1.17	-34.85	-137.05	5.64	-227.24	10.45
d4	-16.27	7.95	-19.44	-110.14	5.87	-29.06	10.99
d5	-16.98	1.44	-20.68	-195.05	7.6	-60.82	14.24

	E (kcal/mol)	RMSD (Å)
Nsp3 on Nsp4	-244.14	3.71
Peptide on Nsp4	-201.89	2.53
Dendron peptide on Nsp4	-141.53	15.72